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Progress Report on Field Surveys to Identify Biocontrol Agents of *Hydrilla verticillata* from June – September 2012

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SUMMARY: Surveys to identify insect herbivores of *Hydrilla verticillata* (L.f.) Royle (hydrilla) (Hydrocharitaceae) were conducted in northern and western regions of Hubei Province and in Jiangxi and Guangdong Provinces from June through November 2012. *Bagous* sp. 2 weevils were collected in Suizhou from hydrilla and *Hydrocharis dubia* (Blume) Backer. Host preference testing and attempts at colonization are underway at the Chinese Academy of Sciences (CAS) laboratories in Wuhan. *Bagous* rufipennis Egorov & Grachev weevils (*Bagous* sp. 3 in previous reports) have been colonized at the United States Department of Agriculture (USDA), Agricultural Research Service (ARS) Australian Biological Control Laboratory (ABCL) in Brisbane, Australia, where host range testing has been initiated. Moths were common in Guangdong Province but appear to be the generalist *Parapoynx diminutalis* Snellen. Surveys will recommence in 2013 during the spring and summer period in Hubei Province and other southern China provinces.

INTRODUCTION: Hydrilla is a submersed aquatic macrophyte that is native to Asia, Australia, Europe, and Africa (Buckingham and Bennett 1998). It was introduced into North America in the early 1950s and has since become highly invasive (Schmitz et al. 1991) where it hinders navigation, impacts water intake and delivery systems, limits recreational uses, out-competes native vegetation, acts as a breeding ground for mosquitoes, and destroys fish and wildlife habitats.

Hydrilla is typically managed through the use of chemical control. Continuous use of a single herbicide has led to the development of resistance to the systemic herbicide fluridone (Michel et al. 2004). The introduction of the herbivorous fish *Ctenopharyngodon idella* (grass carp) can remove hydrilla effectively, but this fish also feeds on a wide variety of desirable native plant species (Hanlon et al. 2000). Mechanical control is expensive and ineffective for the long-term control of this weed. Worldwide surveys for insect biological control agents of hydrilla led to the release of two weevil species (*Bagous hydrillae* O'Brien and *Bagous affinis* Hustache) and two leaf-mining flies (*Hydrellia pakistanae* Deonier and *Hydrellia balciunasi* Bock) (Buckingham and Grodowitz 2004) in the United States, of which only the two *Hydrellia* established. The leaf-mining fly *H. pakistanae* is widespread and damaging, but its impact on hydrilla in the field is limited (Doyle et al. 2002, 2007; Grodowitz et al. 2003; Owens et al. 2006, 2008), and additional agents are required.

Despite worldwide exploration for biological control agents of hydrilla over the last 40 yrs (Balciunas et al. 2002), regions where dioecious hydrilla biotype is native in Southern China and Southeastern

Asia have been poorly surveyed. Since 2006, renewed surveys in China (Figure 1) have found four *Bagous* weevils feeding on hydrilla. *Bagous chinensis* Zumpt was found in Hunan Province in 2007, but host range studies indicated it could develop on four other aquatic plant species: *Egeria densa* (Planch.), *Elodea nuttallii* (Planch.) H. St. John, *Hydrocharis morsus-ranae* L., and *Vallisneria natans* (Lour.) H. Hara (Ding et al. 2009). Only agents specific to hydrilla are suitable to prevent damage to native plants if they are used as a biological control agent in the United States. *Bagous* sp.1 was collected on the Lijiang River in Guangxi Province and was also not specific, developing on *Lagarosiphon alternifolia* (Roxb.) Druce, *E. nuttallii*, *Najas marina* L., and *N. minor* All. (Zhang, unpublished data[1]). *Bagous* sp.2 was collected from Hemufan Village in Suizhou, Hubei Province, from 2009 to 2011 (Ding et al. 2011; Zhang et al. 2012), although failed to be reared. *Bagous rufipennis* was also collected from Suizhou from Lijiafan Village; however, it developed on other macrophyes, *E. nuttallii* and *L. alternifolia* in host-range testing. This report details the results of the field surveys and laboratory tests of insect herbivores on hydrilla in China and Australia from June through November 2012.

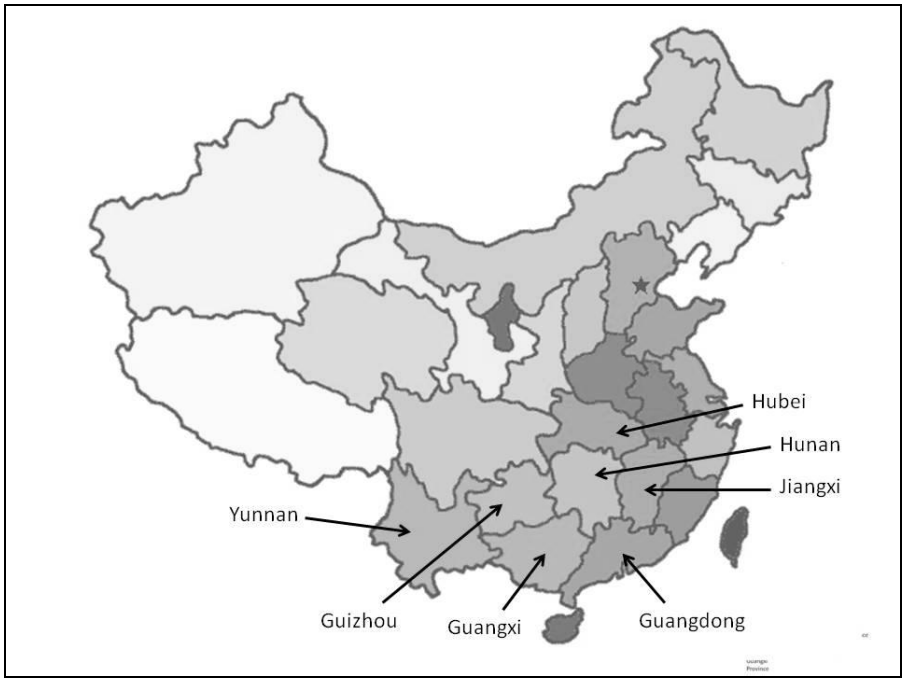

Figure 1. Provinces in southern China where hydrilla was surveyed for insect herbivores.

PROJECT UPDATE

Taxonomy. In January 2013, Dr. Charles O'Brien, an eminent weevil taxonomist in the United States, identified two of the previously unnamed *Bagous* weevils from China. The weevil species

[1] Invasion Biology and Biocontrol Lab, Wuhan Botanical Institute, CAS

named previously as *Bagous* sp. 3 and collected from hydrilla at Lijiafan Village is *Bagous rufipennis* (Figure 2). A weevil collected from *Hydrocharis dubia* at the same site is *Bagous picturatus* Egorov & Grachev (Fig. 3). Visually it is very similar to *Bagous* sp. 2 collected from hydrilla and *Hydrocharis dubia* at Hemufan Village but can be distinguished by its inflated tarsal segments (Figure 3), possibly as an adaption to walking on large surface leaves of aquatic plants. *Bagous* sp. 1 from the Li River in Guangxi, *Bagous* sp. 2, and the unknown *Bagous* collected by Willey Durden in Hebei Province are yet to be named. A large *Bagous* species collected from Western Australia in 2009 was identified as *Bagous natator* O'Brien. There are no published host records for any of the identified species.

Figure 2. *Bagous rufipennis* from Lijiafan.

Figure 3. *Bagous picturatus* from Hemufan.

Survey Areas and Sample Processing. Four surveys were conducted to find potential biological control agents of hydrilla in northern and western regions of Hubei Province from June through September 2012. Hydrilla and associated aquatic plant species (~ 1 kg each, if available) were collected by hand from the shore or while wading and placed into zip-lock plastic bags. Basic site data were recorded for each collection including prevailing weather, location, identity and abundance of all aquatic plant species, and a habitat description as well as sampling point depth and distance from shore. Plant samples were placed in a cooler or car refrigerator for transportation to the laboratory in Wuhan. Non-hydrilla aquatic plant species were collected to determine the field host specificity of insect herbivores. Portions of samples were inspected under a dissecting microscope to observe insect herbivores and associated damage. The balance of the sample was slowly dried in portable Berlese extraction funnels to collect remaining insect herbivores. This technique is particularly effective in extracting internal feeding immatures not easily observed though visual inspection or under a microscope. When possible, immatures were reared to adults. Adults were used for colonization and laboratory testing or preserved for later identification.

Collections in Hubei province. Collections were made at four sites in Hubei Province: Hemufan (Figure 4) and Lijiafan Villages in Suizhou and at Enshi and Yichang cities (Table 1). Three surveys were made to Hemufan and one at Lijiafan along the Fuhe River in Suizhou. However, a survey to Enshi and Yichang in July failed to locate any hydrilla due to heavy rainfall and flooding.

Figure 4. Hydrilla was collected at Hemufan Village, Hubei Province

In the first survey at Hemufan Village in June, only one larva of *Bagous* sp.2 was collected from hydrilla, which died once transferred to fresh hydrilla in the laboratory. In August 2012, *Bagous* sp.2 were collected from both hydrilla (8 adults and 14 larvae) and from *H. dubia* (5 adults and 89 larvae). No adults or larvae were extracted from *N. marina* and *Ceratophyllum demersum L. collected in the same survey*. In September, 3 adults and 26 larvae were collected from hydrilla, 2 adults and 6 larvae from *H. dubia,* and 12 adults and 250 larvae from *C. demersum*. Other herbivores collected included *Parapoynx* spp., Chironomidae larvae, and Elmidae (Table 1). In September, the ponds at Lijiafan Village were covered with *H. dubia*, and no hydrilla could be found. Two adults and more than 60 larvae of *Bagous pictoralis* were extracted from an *H. dubia* sample.

Exploratory surveys were conducted in northern Hubei Province at Hemufan Village in November 2012. No *Bagous* weevils were extracted from hydrilla collected at this site though the weather was very cold and the plant material was in poor condition (Figure 5). *Bagous* sp. 2 is usually collected from this site and is currently undergoing host range testing at the Chinese Academy of Sciences (CAS) Wuhan.

Table 1. Field sites surveyed in Southern China for insect herbivores of *Hydrilla verticillata* in 2012, including location, collection date, and numbers of adults or larvae observed on *H. verticillata* and associated aquatic plant species.

Province	Site	GPS	Collection date	Host Plant	Herbivore	Number/Stage	
						Adults	Larvae
Hubei	Lijiafan Village	N31°26′ E113°34′	18-19 Sep 2012	*Hydrocharis dubia*	*Bagous pictoralis*	2	60
	Hemufan Village	N31°46′ E113°07′	26-27 Jun 2012	*Hydrilla verticillata*	*Bagous* sp.2		1
					Parapoynx sp.		7
			24-25 Aug 2012	*Hydrilla verticillata*	*Bagous* sp.2	8	14
					Parapoynx sp.		57
					Chironomidae		147
					Elmidae	4	12
				Hydrocharis dubia	*Bagous* sp.2	5	89
					Parapoynx sp.		3
			18-19 Sep 2012	*Ceratophyllum demersum*	*Bagous* sp.2	12	250
					Chironomidae		3
				Hydrilla vertcillata	*Bagous* sp.2	3	26
					Parapoynx sp.		6
					Chironomidae		25
				Hydrocharis dubia	*Bagous* sp.2	2	6
					Parapoynx sp.		29
					Chironomidae		154
			3 Nov 2012	*Ceratophyllum demersum*	Nil		
				Hydrilla vertcillata	Nil		
				Vallisneria natans	Nil		
	Enshi City	N30°16′ E109°29′	11-15 Jul 2012	No aquatic plants, flooding			
	Yichang City	N30°12′ E110°40′	11-15 Jul 2012	No aquatic plants, flooding			
Guanagdong	Chetian Town	N24° 26’ E115° 17’	8 Nov 2012	*Hydrilla verticillata*	*Parapoynx* sp.		30
					Chironomidae	1	2
	Lizui Town	N24° 21’ E115° 19’	8 Nov 2012	*Hydrilla verticillata*	*Parapoynx* sp.		5
					Chironomidae		3
	Pengzhai Town	N24° 21’ E115° 05’	9 Nov 2012	*Hydrilla verticillata*	*Parapoynx* sp.		47
					Chironomidae		7
	Linzhai Town	N24° 17’ E115° 07’	9 Nov 2012	*Hydrilla verticillata*	*Parapoynx* sp.		17
	Chuantang Town	N24° 11’ E114° 57’	9 Nov 2012	*Hydrilla verticillata*	Nil		

Figure 5. Hydrilla was in poor condition at Hemufan due to cold water temperatures.

Collection in Jiangxi and Guangdong. Surveys were also conducted in Jiangxi and Guangdong Provinces. No hydrilla was found in Jiangxi, but samples were collected at Chetian Town, Lizui Town, Pengzhai Town, Linzhai Town, and Chuantang Town in Guangdong (Table 1). No insect herbivores were collected from hydrilla at Chuantang. At the remaining sites, a large number of moth larvae were extracted from samples, particularly from Pengzhai Town (Figure 6). Adults have been reared for identification, but most appear to be the generalist *Parapoynx diminutalis*. Specimens will be sent to taxonomists for full identification. Chironomid larvae damaging tips and emerging adults were collected from hydrilla at Chetian Town, Lizui Town, and Pengzhai Town. No *Bagous* weevils were extracted from these samples.

LABORATORY REARING AND HOST RANGE TESTING

***Bagous* sp.2.** The aquatic weevil *Bagous* sp.2 (Figure 7) was a high priority for research given that they pupated in cocoons on the plant, possibly in the water. All other *Bagous* weevils found on hydrilla appear to pupate only along the shoreline, usually in soil and desiccating plant material. Completing development on the plant is highly desirable given hydrilla's prevalence in open water including large lakes and rivers. This weevil has consistently been collected from a bridge site near Hemufan Village in Suizhou City, Hubei Province, since 2010 (Zhang et al. 2012). Field hosts include hydrilla, *C. demersum,* and *H. dubia*.

Figure 6. Large numbers of moth larvae were collected at Pengzhai Town.

Figure 7. Mating adults of the aquatic weevil, Bagous sp. 2.

In the laboratory, field-collected mature larvae of *Bagous* sp.2 were placed onto hydrilla and *H. dubia* in containers with and without water. Although some mature larvae died, some pupated on the leaves of the plant, though naked with cocoons absent. Initially, some of the larvae fed on both plant

species. In total, 41 adults have been reared from both plant species in containers with and without water. Host range testing has not been completed, and plant species under evaluation include *Brasenia schreberi* J.F. Gmel., *Cabomba caroliniana* A. Gray, *C. demersum*, *H. dubia*, *Myriophyllum verticillatum* L., *Najas marina* L., *Potamogeton malaianus* Miq., *Vallisneria natans* (Lour.) Hara, and *Potamogeton crispus* L.

***Bagous rufipennis*.** Efforts at ABCL in Brisbane, Australia, focused on rearing a substantial culture of *Bagous rufipennis* from the small numbers of adults hand carried from China in 2011. The quarantine culture now contains in excess of 200 weevil adults. As a result, host range testing was initiated in September 2012. In each trial, 5–10 g of a single plant species (including stems, leaves, and roots, where possible) were placed in a 20 cm × 20 cm × 12 cm square plastic food container lined with moist filter paper. Twenty adult *B. rufipennis* were released into the container for 7 days. Plant material was replaced after 3–4 days. Each test had at 1–2 test plant species and a hydrilla control (2–3 containers). After 7 days, the adults were removed, and the plant material was set aside for development of immatures; fresh plant material was added as necessary. Tests have been conducted on five aquatic plant species, and *B. rufipennis* was reared on hydrilla as well as *Vallisneria spiralis* L. and *Egeria densa* (Planch.) Casp. Results of these tests are given in Table 2. Testing is ongoing and will be finalised in 2013. *Bagous sp. 2* adults were also imported from China into ABCL quarantine in November 2012. As yet, these weevils have failed to oviposit on hydrilla. These adults were collected from both hydrilla and *Hydrocharis dubia* at Hemufan Village, so their identification may be incorrect given that the third species from *Hydrocharis dubia*, *Bagous picturatus*, has also been collected in the region. Now that distinguishing taxonomic characters have been obtained from O'Brien to separate the species, colonies will be verified as being a single species.

Table 2. Results of no-choice host range tests of Bagous rufipennis conducted in ABCL quarantine in Brisbane.

Plant Species	No. Tests	No. Valid Tests	No. Adults Reared	Mean Adults Reared per Test
Hydrilla verticillata	6	5	18	3.6
Vallisneria spiralis	4	3	3	1
Egeria densa	3	3	12	4
Myriophyllum aquaticum (Vell.)	3	3	0	0
Ottelia ovalifolia (R. Br.) Rich.	2	1	0	0

FUTURE PLANS

Exploration

- Expansion of field surveys in Yunnan, Guizhou, and Hubei Provinces are needed to identify new, potential biological control agents of hydrilla. Sites in Yunnan and Guizhou have excellent relatively natural and undisturbed areas, which appear to have excellent diversity of flora and fauna.
- Two, new stem-boring *Bagous* weevils have recently been discovered in Hubei, and further exploration in this province is warranted.
- Continued exploration will be conducted in Guangxi Province to find new agents.

Rearing, biology, and host specificity

- Conduct field host range surveys of *Bagous* sp.2 and *B. rufipennis* at hydrilla field sites in Hubei Province.
- Establish a colony of *Bagous* sp.2 from Hubei Province and evaluate whether hydrilla is a true host of this weevil and verify whether its development can be completed underwater.
- Complete host range testing of *B. rufipennis.*

Taxonomy

- Curate and catalogue all insect herbivores identified in exploratory field surveys and send to appropriate taxonomists for identification.
- Collection sites will be checked for the presence of dioecious and monoecious hydrilla, particularly the source sites where *Hydrellia balciunasi* was collected in Australia for U.S. testing and field releases.

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